can be estimated from the difference in ratios:

$$\left[C^{14}\right]_{\substack{\text{excess}\\ \text{in free}\\ \text{metanephrine}}} = \left(\left[\frac{C^{14}}{H^3}\right]_{\substack{\text{free}\\ \text{meta-}\\ \text{neph-}\\ \text{rine}}} - \left[\frac{C^{14}}{H^3}\right]_{\substack{\text{conju-}\\ \text{gated}\\ \text{meta-}\\ \text{neph-}\\ \text{rine}}}\right)\left[H^3\right]_{\text{free}}$$

The correction of the injected metanephrine C^{14}, by subtraction of the excess urinary free metanephrine-C^{14}, is necessitated by the initial rapid excretion of the injected compound. In order to be conjugated or metabolized, however, the injected metanephrine-C^{14} must enter the tissues. It is assumed that the lag in the initiation of these reactions is similar and that metanephrine and epinephrine can enter the various tissues with equal ease, so that a correction for conjugated metanephrine is unnecessary.

Using these principles, I made an attempt to evaluate the pathways of metabolism of epinephrine in man. Two normal males received 38.4 μc of L-epinephrine-7-H^3 and 4.58 μc of L-metanephrine-methoxy-C^{14} (*6*), and the various metabolites were isolated (*5*) from the urine collected during the following 48 hours. The amounts of H^3 and C^{14} in each compound were determined simultaneously in a liquid scintillation counter (*2*). Table 1 indicates the distribution of radioactivity in the various compounds isolated from the urine. From the ratios of H^3/C^{14} found for each compound in the equations outlined, it was calculated that 66.1 percent and 68.0 percent of the injected epinephrine was methylated to form metanephrine. Of this, 20 percent and 21.4 percent formed VMA and 2.25 percent and 3 percent formed MHPG. Since 44.4 percent and 45.2 percent of the injected epinephrine formed VMA, 24.4 percent and 23.8 percent must have been formed by deamination followed by methylation. Similarly, 2.25 percent and 3.3 percent of the MHPG was formed by deamination followed by methylation. A total of 95.8 percent and 98.1 percent of the injected epinephrine could be accounted for by methylation to metanephrine, deamination prior to methylation to form VMA and MHPG, and excretion as unchanged or conjugated epinephrine.

The use of simultaneous labeling of two different metabolites in determining the relative importance of alternate pathways of metabolism can be widely applied (*7*).

IRWIN J. KOPIN
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland

References and Notes

1. See, for example, E. H. LaBrosse, J. Axelrod, S. S. Kety, *Science* **128**, 593 (1958).
2. G. T. Okita, J. J. Kabara, F. Richardson, G. V. LeRoy, *Nucleonics* **15**, 111 (1957).
3. H. Blaschko, D. Richter, H. J. Schlossman, *J. Physiol. (London)* **90**, 1 (1937); C. E. M. Pugh and J. H. Quastel, *Biochem. J.* **31**, 256 (1937); D. Richter, *ibid.* **31**, 2022 (1937).
4. J. Axelrod, *Science* **126**, 400 (1957).
5. D. Richter, *J. Physiol.* **98**, 361 (1940); M. D. Armstrong, A. McMillan, K. N. F. Shaw, *Biochim. et Biophys. Acta* **25**, 442 (1957); J. Axelrod, S. Senoh, B. B. Witkop, *J. Biol. Chem.* **233**, 697 (1954); J. Axelrod, I. J. Kopin, J. D. Mann, *Biochim. et Biophys. Acta*, in press.
6. A description of the preparation of these compounds is being written.
7. I would like to express my appreciation to Dr. Seymour S. Kety for his interest and encouragement, as well as his suggestions, in the development of this method.

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On the Thermal Boundary Layer of the Ocean

Abstract. Measurement of the long-wave infrared radiation from the top 0.1 mm of the evaporating ocean demonstrates the existence of a cool surface layer characterized by departures of as much as 0.6°C from the "surface temperature" found by conventional methods. Being very thin, the layer cools sufficiently rapidly to reestablish itself in less than 12 seconds after disruption by a breaking wave.

By means of simultaneous measurements of the radiation temperature and of the conventional thermometric temperature of the ocean, we have found evidence of a persistent cool boundary layer. The equipment used, shown in Fig. 1*A*, consisted of a double-beam radiometer having a spectral sensitivity in the band from 6 to 20 μ, a region in which the absorption in water is so high that 98 percent of the radiant flux originates in the first 0.1 mm. To minimize the necessary corrections for absorption by air and for reflection, the measurements were made at normal incidence at night from a position 2 m above the water. The water was shaded as required. For comparison, the temperature of the water beneath the radiation layer was measured by thermistors encapsuled in 1-mm glass beads, at depths dictated by the surface conditions of swell, waves, and ripples. The system had over-all sensitivity sufficient to discriminate temperatures with an uncertainty of less than 0.1°C, with response of less than 1 second.

Ocean measurements were made from the Scripps pier at a point 200 m off shore in water 7 m deep. A sample traced from the data is shown in Fig. 1*B*, which includes, for comparison, the "surface temperature" obtained by thermistor. The latter was checked by bucket sampling and mercury thermometer. The radiation temperature, when the ocean was shaded from the clear, cold night sky, was more than 0.7°C lower than the thermistor temperature. Since the screen used was at air temperature and therefore cooler than the ocean, a correction of +0.1°C must be applied to the measured value, which leaves a departure of the radiation temperature of more than −0.6°C. It should be noted that the conditions of wind and humidity were not conducive to vigorous evaporation. The effect of exposing the ocean to the night sky by removing the shade is readily seen in the right-hand section of the figure.

In order to evaluate the effect of breaking waves, a small electric rotary pump was submerged beneath the radiometer, positioned so as to draw water from a depth of 15 cm and direct it as a jet which welled up in the radiometer's field of view. It was determined by bathythermogram and thermistor that the water below the upper centimeter was isothermal within measurable limits. The result of intermittent pumping is shown in Fig. 1*C*. When the pump was run sufficiently vigorously to rupture the surface in the manner of a bubbling spring, the radiation temperature rose to approximately the values measured by the thermistor submerged at the level of the pump intake. When the pump was shut off, the radiation temperature dropped to its normal value in about 5 seconds, the cooling rate indicating that the effect takes place in a layer less than 1 mm thick. A remarkable finding was that less intense disturbance of the water failed to produce measurable effects.

Radiometric measurement over a breaking wave gave a concordant result. Coincident with the breaking, a momentary small warm signal was recorded, followed by a longer-lasting, stronger cold signal which seemed to coincide with the life span of the blanket of foam left behind by the wave. The whole disturbance lasted about 12 seconds; then the radiation returned to its normal value. Thus, on the open sea where whitecaps occur at a given point at relatively long intervals, the thermal boundary layer should be present, at least intermittently.

The chief features observed on the ocean were modeled in a controlled laboratory environment. In Fig 1*D* is shown, at the left, the radiation temperature of a salt-water surface being gradually warmed by radiation from the ceiling and walls of the room. Under these conditions, the heat flux was downward, and the water was initially in a state of stable stratification with a warm surface layer. As indicated in the figure, a fan was caused to blow periodically on the surface, the air stream having a velocity of about 1 m/sec and a relative

humidity of 55 percent. At the onset of evaporation, the radiation temperature quickly dropped about 1°C in 40 seconds, the polarity of its departure from the bulk temperature reversing from positive to negative. Cessation of the draft produced a return to the initial temperature but at a much slower rate, due, no doubt, to the diminished role of convection in the flow of heat. At the right side of the figure is shown the result of vigorous agitation by a conventional laboratory stirring device positioned so as to produce strong vertical currents in the water. The precipitous drop in temperature coincided with the arrival of cool subsurface water in the radiation layer. Increased evaporation caused by the fan while the stirring continued is plainly shown in the record, though the temperature departures are much reduced in amplitude, probably because of induced changes in the thermal conductivity of the boundary layer.

Additional laboratory experiments were performed with varying conditions of imposed radiation, evaporation, and stability. The general result was that the departure of the radiation temperature from the bulk temperature increased with the flux of heat through the radiation layer, the polarity being positive for downward flow and negative for upward flow. Thermal conditions were steadier in the case of downward heat flux. Moderate vertical stirring reduced but did not obliterate the thermal boundary layer.

It is well known that the ocean, in ice-free latitudes, is heated to a considerable depth by short-wave solar radiation, the heat balance being largely maintained by evaporation and long-wave back radiation from much shallower depths. It follows, therefore, that the heat flux in the superficial layers must, on the average, be upward. Consequently, one may conclude that the radiation temperature of the ocean and other natural bodies of water is usually lower than the ordinary surface temperature.

Because the thermohydrodynamics of a free saline liquid surface have not, as yet, been formulated in detail, it would be premature to attempt a theoretical analysis of the phenomena observed. Neither would it be prudent to identify the phenomena observed too closely with phenomena characteristic of rigid-boundary surfaces. Nevertheless, the experimental results appear consistent with the hypothesis that a boundary layer exists, immediately under the ocean surface, in which transfer of sensible heat is controlled chiefly by molecular conduction rather than by convection or turbulent exchange. In this layer, thermal gradients are maximized. The transition between the boundary layer and the deeper region where turbulent conduction is fully developed is characterized by unsteady thermal conditions which we observed by placing thermistors in vertical arrangement near the surface. Fluctuations appeared, having time constants of less than 1 second. The unsteadiness is much more fully developed when the heat flux is upward than when it is downward. Temperature fluctuations in air heated close

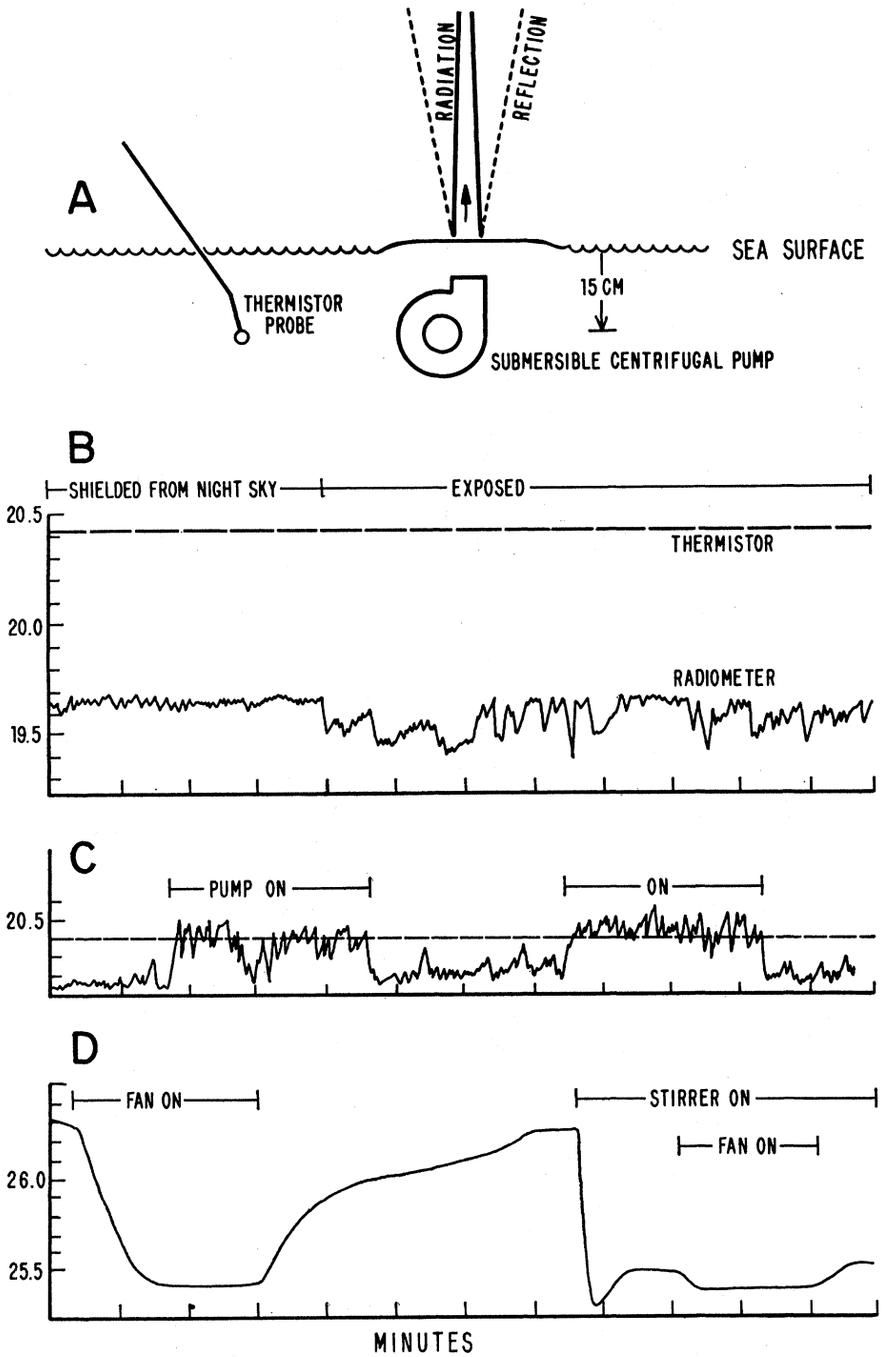

Fig. 1. Simultaneous measurements of the radiation temperature and of the subsurface temperature of evaporating water surfaces. (*A*) Schematic diagram of apparatus. (*B*) Trace of radiometer record in relation to thermistor temperature (dashed line), 2 October, 2300 (P.S.T.); wind, at 10 m above mean sea level, ½ m/sec; sky clear; air temperature 2 m above mean sea level, dry bulb 18.4°C, wet bulb 16.7°C; air temperature 8 m above mean sea level, dry bulb 18.0°C, wet bulb 16.2°C; relative humidity, 83.5 percent at 2 m, 85.0 percent at 8 m. (*C*) Effect of water jetted into surface by submerged pump. (*D*) Effect of induced evaporation and mechanical stirring on water in an insulated container. Air temperature, 32.8°C; fan velocity, 1 m/sec; relative humidity, 55 percent.

to the ground are described in some detail by Sutton (*1*) being attributed to bubbles of overheated air which periodically detach themselves from the thermal boundary layer. In a personal communication, R. W. Stewart, described similar phenomena observed by means of the schlieren method in the cool, unstable thermal boundary of evaporating water. Unsteady thermal conditions in the surface of cooling ponds and sheltered estuaries have been reported by Woodcock and Stommel (*2*).

Further study of the thermal boundary layer should reveal details of the mechanism by which contaminating surface films reduce evaporation from natural bodies of water. In general, it is to be noted that surface temperatures of the ocean or other wind-swept bodies of water determined by conventional methods are systematically biased in a positive sense (*3*).

GIFFORD EWING
Scripps Institution of Oceanography, University of California, La Jolla
E. D. MCALISTER
Apparatus and Optics Division, Eastman Kodak Company, Rochester, New York

References and Notes

1. O. G. Sutton, *Micrometeorology* (McGraw-Hill, New York, 1953), chap. 4.
2. A. H. Woodcock, *J. Marine Research* (*Sears Foundation*) **4**, 153 (1941);——— and H. Stommel, *J. Meteorol.* **4**, 102 (1947).
3. This study was supported by the Office of Naval Research. Credit is due Norman E. Head for technical assistance. This report is a contribution from the Scripps Institution of Oceanography.

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New Method for Manipulation, Maintenance, and Cloning of Single Mammalian Cells in vitro

Abstract. Individual mammalian cells can be isolated with the aid of easily handled glass beads to which the cells have become attached. Procedures for the preparation of cell cultures on beads, and for the recognition and manipulation of beads carrying single monocytes, HeLa cells, and Detroit-98 cells are described. Data obtained with *Brucella* infected monocytes, illustrating the efficiency of the method, are presented.

During studies of interactions between *Brucella abortus* strains of different virulence and mammalian monocytes maintained in vitro (*1*), it became apparent that certain problems—specifically, those associated with possible interference phenomena affecting continued ingestion of virulent brucellae—required an analysis of interactions involving single phagocytes. This requirement led to the development of a novel and relatively simple method for isolating single monocytes: exploratory trials indicate that this technique may also be used for isolating other mammalian cell types. The principal feature of the new method is the fixing and manipulation of single cells that have been permitted to adhere to the surface of small glass beads (*2*) partially embedded in a paraffin (or 2 percent agar) layer contained in small glass cups.

Table 1. Distribution of brucellae per monocyte among bead isolates and stained monocytes, determined by plating single monocytes and counting the resultant colonies or by the microscopic observation of stainable intracellular brucellae. Each figure in this tabulation indicates the number of monocytes containing, according to the column in which they are recorded, 0 to more than 10 bacteria.

No. of brucellae per monocyte											
0	1	2	3	4	5	6	7	8	9	10	>10	Total
Plating bead isolates												
11	5	3	6	4	4	2	0	0	1	0	4	40
Stained coverslip No. 1												
12	7	5	4	3	3	2	0	1	0	0	3	40
Stained coverslip No. 2												
9	9	6	6	3	2	1	1	0	1	1	1	40
Stained coverslip No. 3												
10	7	7	5	6	2	0	0	1	0	0	2	40

Before exposure to cell suspensions, the beads are boiled in detergent, washed in distilled water, dried, and sterilized by dry heat. They are then placed in sterile glass cups (5 ml Beckman *p*H-meter cups, cut to a height of approximately 5 mm) containing a thin layer of molten Vaspar (equal volumes mixture of noncarbolated Vaseline and household paraffin). The Vaspar layer is prepared by adding to thoroughly dried, heated cups a small amount of hot (about 100°C) Vaspar, the cups being immediately inverted to remove excess Vaspar. To the liquid Vaspar layer remaining within the cups, beads (about 500 per cup) are added by gentle sprinkling, which results in an even distribution of well-separated beads anchored in the Vaspar layer to about half their depth. Slow cooling of the bead-seeded cups is important and is achieved by placing the cups in heated petri dishes.

To isolate single monocytes, cells are harvested by previously described methods (*3*), and 0.8 ml of an appropriate suspension (containing approximately 5×10^4 monocytes per milliliter in a mixture of autologous serum and Hanks' solution) is added to each cup. The cups are sealed with silicone-greased 1-inch square coverslips, to prevent loss of carbon dioxide and water, and incubated at 37°C. After approximately 2 hours, the cups are examined for the presence of beads with single monocytes on their surface. Such beads may be removed for immediate examination or may be permitted to remain in the cups for any desired period of time.

The recognition of single monocytes on individual beads requires careful microscopic examination using proper lighting. We routinely employed a dissecting microscope with 18× ocular and 6× objective, using a sheet of green blotting paper in place of the usual substage mirror. When properly illuminated, monocytes adhering to the exposed bead surface are visible as differently refracting, irregularly-shaped cells, whereas the air bubbles found in most beads are seen as highly refractive circles.

For removal of the beads from their Vaspar bed, capillary pipettes with a diameter slightly greater than that of the beads were used. Beads were picked up by punching out a single bead plus its Vaspar base, and were removed from the pipette by tapping or by positive pressure.

The efficiency of the method is illustrated by a comparison of the distribution of numbers of brucellae in individual monocytes as determined by the bead method and by direct microscopic examination of stained monocytes (Table 1). For the former procedure, monocytes, infected with brucellae 30 minutes earlier, were introduced into the cups in the presence of streptomycin to kill all extracellular bacteria. Two hours later beads carrying single monocytes were removed and lysed on the surface of nutrient agar with the aid of 2 percent saponin (*4*), and the number of intracellular bacteria was determined by counting the colonies that developed after 5 days. For the procedure employing direct examination, infected monocytes were allowed to adhere to flying-coverslips in the presence of streptomycin, and were stained 2 hours later by Machiavello's technique (*5*). The data in Table 1 indicate a close correlation between the results obtained by these two procedures.

The method of isolating single cells